

OHIO ASCOMYCETE NOTES

II. TALAROMYCES FROM SOILS OF SOUTHERN OHIO¹

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HUANG, L. H. AND J. A. SCHMITT. Ohio Ascomycete Notes II. *Talaromyces* from soils of southern Ohio. Ohio J. Sci. 75(2): 75, 1975.

Seven species of *Talaromyces* were isolated from 14 soil samples from southern Ohio. Of these, *T. helicus* var. *helicus*, *T. luteus*, and *T. udagawae* are rare in soils. *Talaromyces luteus* occurred in eight different localities in southern Ohio. *Penicillium ucrainicum* Panasenko is synonymous with *T. flavus* var. *flavus*, and the name *P. ohioensis* Huang & Schmitt is proposed for the imperfect state of *T. ucrainicus* Udagawa. These organisms were discovered through use of the alcohol treatment technique which appears to be highly effective for isolating ascocarpic *Penicillium* from soil.

The genus *Talaromyces* was established by Benjamin (1955) to accommodate the known ascomycetous forms with ascocarpic peridium consisting of loosely interwoven hyphae and with a *Penicillium* imperfect state. Stolk and Samson (1971) transferred *Talaromyces avellaneus* and *T. striatus* to a new genus *Hamigera* on the basis that the asci in these two species are produced from croziers instead of in chains as is characteristic of other *Talaromyces* species. In a monographic study Stolk and Samson (1972) redefined the genus *Talaromyces* to include species producing asci in chains and having *Penicillium* or *Paecilomyces* as the imperfect state. They described two new species and two new varieties, and recognized sixteen species and four varieties divided into four sections: *Talaromyces*, *Emersonii*, *Thermophila*, and *Purpurea*. Of the 16 species, *Talaromyces byssochlamydoides*, *T. emersonii*, *T. leycettanus*, and *T. thermophilus* are known to be thermophilic (Evans and Stolk, 1971; Stolk, 1965; Stolk and Samson, 1972).

This paper deals with seven species of *Talaromyces* encountered while surveying the soil fungal flora of Ohio. An attempt is made to clarify nomenclatural problems

with regard to some species of *Talaromyces*.

MATERIALS AND METHODS

Twenty soil samples from southern Ohio were collected in July and August, 1972. Following collection, the samples were refrigerated at 3°C. Isolations were made two months later by a modification of Warcup and Baker's (1963) alcohol treatment technique. Half-gram samples of soil were steeped in 65% ethanol for 12 minutes. The liquid was then decanted, bits of the treated soil dispensed into 15 sterile petri dishes, and the plates immediately poured with Gochenaur's (1964) glucose ammonium nitrate agar. The latter contains rose bengal to reduce fungal colony spread and streptomycin to inhibit bacteria. The dishes were gently rotated to disperse the soil particles before the agar solidified, then were placed in a dark wooden cabinet for incubation at room temperature. For study in pure culture, the *Talaromyces* isolates obtained were grown on Dodge's cornmeal (CM) (Grosklags and Swift, 1957), Czapek-Dox (Cz), and malt extract (M) (Raper and Pennell, 1965) agars. Incubation was at room temperature and under ordinary laboratory conditions of alternating darkness and light. Roman numerals in parentheses refer to plates in Ridgway's Color Standards (Ridgway, 1912).

Collection sites of Ohio soil samples were as follows:

- A. Adams Co., 1 mi west of Lynx.
- B. Adams Co., 1 mi east of West Union.
- C. Adams Co., 1 mi west of West Union.
- D. Athens Co., 14 mi west of Athens.
- E. Athens Co., 5 mi east of Athens.
- F. Belmont Co., 4 mi southeast of Belmont.
- G. Belmont Co., north Dysart Woods near Belmont.
- H. Belmont Co., south Dysart Woods near Belmont.
- I. Clark Co., 3 mi north of Springfield on US 68.
- J. Clermont Co., 1.5 mi east of Bethel.
- K. Franklin Co., 1 mi south of Columbus on US 23.
- L. Muskingum Co., 10 mi northwest of Zanesville on SR 146.
- M. Ross Co., 9 mi northeast of Chillicothe.
- N. Scioto Co., 2 mi northeast of Portsmouth.

Cultures of *Talaromyces ucrainicus* NHL 6086 (from Udagawa) were grown for comparison.

RESULTS

Fourteen of 20 soil samples yielded *Talaromyces*, giving a total of seven spe-

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cies. *Talaromyces luteus* and *T. trachyspermus* occurred in 8 localities, whereas *T. helicus* var. *helicus* and *T. ucrainicus* each were found in a single locality. The other three fungi, *T. flavus* var. *flavus*, *T. wortmannii*, and *T. udagawae*, were isolated from 6, 6, and 2 localities, respectively.

A key to the seven species of *Talaromyces* is provided. This is followed by notes on each species. *Penicillium ucrainicum* Panasenکو is synonymous with *T. flavus* var. *flavus*. *Penicillium ohioensis* Huang & Schmitt is proposed for the imperfect state of *T. ucrainicus* Udagawa.

Key to species of *Talaromyces* in Ohio soils

- 1 Ascospores with ridges or spines. 2
- 1' Ascospores with transverse or spiral bands. 3
 - 2(1) Ascospores with longitudinal or spiral ridges. *T. ucrainicus*
 - 2'(1) Ascospores with delicate spines. 4
- 3(1') Ascospores large, mostly over 4.5 μ m long. *T. luteus*
- 3'(1') Ascospores smaller, mostly under 4.5 μ m long. *T. udagawae*
- 4(2') Ascospores large, mostly over 4 μ m long. 5
- 4'(2') Ascospores smaller, mostly under 3.5 μ m long. 6
- 5(4) Ascocarp initial consisting of an irregular swelling and septation of a hyphal segment. *T. wortmannii*
- 5'(4) Ascocarp initial consisting of a long, clavate hypha. *T. flavus* var. *flavus*
- 6(4') Ascocarp initial consisting of a long, helicoid hypha. *T. helicus* var. *helicus*
- 6'(4') Ascocarp initial consisting of an irregular swelling of a hyphal segment which branches profusely. *T. trachyspermus*

1. *Talaromyces flavus* (Klöcker) Stolk & Samson var. *flavus* Figures 1a-f
= *Talaromyces vermiculatus* (Dangeard) (Benjamin, 1955).

Conidial state:

Penicillium vermiculatum Dangeard
= ?*Penicillium ucrainicum* (Panasenکو, 1964) not validly published, since no type was designated.

Isolate 1601b essentially agreed with the species description given by Stolk and Samson (1972) except that its growth on M was slower (4.3 cm vs 7–8 cm in colony diam. in 10–12 da).

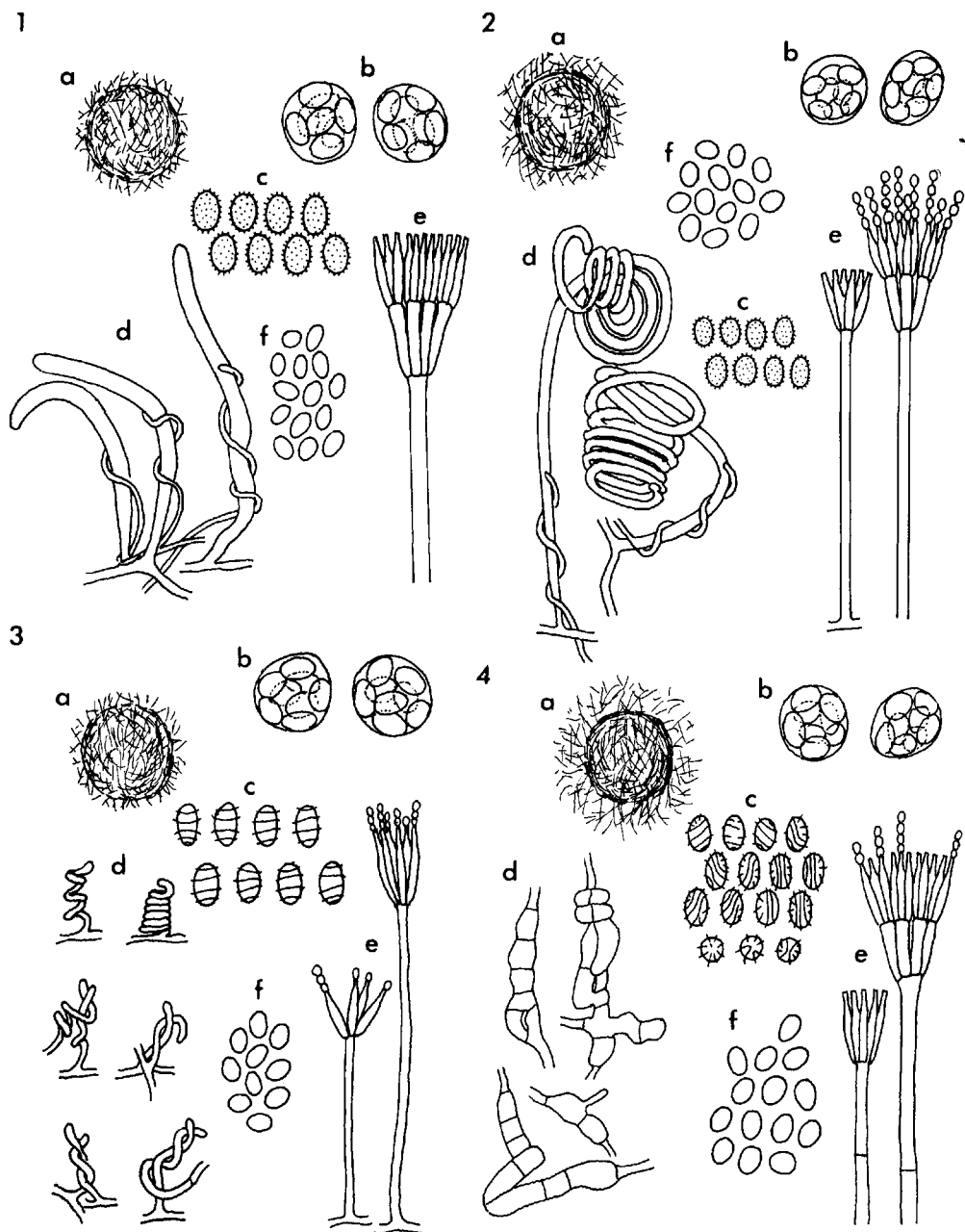
Cultures examined.—Isolated from sites A, B, C, F, I, N.

Cultural variations were observed among strains 1601b, 1701a, 1801b, and 1901b of *T. flavus* var. *flavus*. On Cz, the colony may range from white (1701a), yellowish (1601b, 1801b), to buff (1901b) with the reverse being morocco red (I, 1601b), to yellowish (1901b). The colony reverse on M varies from nopal red (I, 1601b) to pale yellow orange (III, 1701a, 1901b).

There has been controversy concerning the nomenclature of *Penicillium ucrainicum* Panasenکو. Panasenکو (1964) described this fungus from a culture isolated from starch in the Ukraine. In addition to the *Penicillium* imperfect state, he illustrated the perfect state which clearly belongs in the genus *Talaromyces*. Udagawa (1966) studying strains NHL 6086 and 6087 isolated from Japanese soil, concluded that his strains were the same as Panasenکو's fungus, and made the new combination *T. ucrainicus* (Panasenکو) Udagawa. The ascocarp initials illustrated by Udagawa for *T. ucrainicus*, however, are somewhat suggestive of *T. wortmannii*, whereas those illustrated by Panasenکو are identical with ascocarp initials of *T. flavus* var. *flavus*. *Talaromyces ucrainicus* (Panasenکو) Udagawa has been changed to *T. ucrainicus* Udagawa by Stolk and Samson (1972) who regarded Udagawa's fungus as a distinct species in the genus *Talaromyces*. Since the original culture of *P. ucrainicum* has failed to produce ascocarps (personal communication with D. I. Fennell and A. C. Stolk) and since the initials in *P. ucrainicum* and *T. flavus* var. *flavus* are identical, we consider *P. ucrainicum* synonymous with *T. flavus* var. *flavus*.

2. *Talaromyces helicus* C. R. Benjamin var. *helicus* Stolk & Samson
Figures 2a-f
= *Talaromyces helicus* (Raper & Fennell) (Benjamin, 1955). Conidial state: *Penicillium helicum* Raper & Fennell

Isolate 1701b essentially fitted the species description by Stolk and Samson (1972) except that its growth on M was slower than the type (4 cm vs 6 cm in colony diam. in 2 wk).



FIGURES 1a-f. *Talaromyces flavus* var. *flavus*. a. Ascocarp, $\times 50$. b. Asci, $\times 1700$. c. Ascospores, $\times 1800$. d. Ascocarp initials, $\times 1400$. e. Detail of a penicillus, $\times 1400$. f. Conidia, $\times 1800$.

FIGURES 2a-f. *Talaromyces helicus* var. *helicus*. a. Ascocarp, $\times 70$. b. Asci, $\times 2100$. c. Ascospores, $\times 2200$. d. Ascocarp initials, $\times 1500$. e. Detail of penicilli, $\times 1100$. f. Conidia, $\times 1500$.

FIGURES 3a-f. *Talaromyces luteus*. a. Ascocarp, $\times 60$. b. Asci, $\times 1800$. c. Ascospores, $\times 1800$. d. Ascocarp initials, $\times 1400$. e. Detail of penicilli, $\times 1300$. f. Conidia, $\times 1700$.

FIGURES 4a-f. *Talaromyces ucrainicus*. a. Ascocarp, $\times 50$. b. Asci, $\times 2100$. c. Ascospores, $\times 2150$. d. Ascocarp initials, $\times 1700$. e. Detail of penicilli, $\times 1100$. f. Conidia, $\times 2000$.

Cultures examined.—Isolated from site A.

3. *Talaromyces luteus* (Sacc.) Stolk & Samson Figures 3a-f
= *Talaromyces luteus* (Zukal) (Benjamin, 1955). Conidial state: *Penicillium luteum* Zukal

Isolate 1106 slightly differed from the species description given by Stolk and Samson (1972) in its smaller conidia ($2.5\text{--}3.2 \times 2\text{--}2.5 \mu\text{m}$ vs $3\text{--}4 \times 2.2\text{--}3.7 \mu\text{m}$) and yellowish to honey yellow (XXX) rather than mars yellow (III) colony reverse on M.

Cultures examined.—Isolated from sites A, C, D, E, F, I, M, N.

Our observation that ascocarp initials consist of one or two short, coiled hyphae is similar to that by Raper & Thom (1949). That the initials develop usually from atypical phialides, as noted by Stolk and Samson (1972), has not been confirmed. Nor did we find the ascospores with interrupted ridges as noted by Stolk and Samson (1972). Instead, the ascospores of the Ohio strains show transverse, continuous ridges on the surface.

4. *Talaromyces ucrainicus* Udagawa Stud. Mycol. 2: 34. 1972

Figures 4a-f
= *Talaromyces ucrainicus* (Panassenko) (Udagawa, 1966) not validly published since basionym was not validly published.

Conidial state: *Penicillium ohiensis* Huang & Schmitt, sp. nov.

Penicillium ohiensis Huang & Schmitt, sp. nov. Figures 4e-f

Conidiophora laevia vel asperata, 1–3-septata, $30\text{--}80 \times 1.5\text{--}2.5 \mu\text{m}$; penicilli biverticillati, irregulariter biverticillati vel monoverticillati, metulae $10\text{--}14 \times 2\text{--}3 \mu\text{m}$, 2–6 in quoque verticillo; phialides $9\text{--}14$ (–18) $\times 1.8\text{--}2.2 \mu\text{m}$, 2–6-verticillatae; conidia e subgloboso elliptica, laevia, hyalina, $2\text{--}3.5 \times 1.5\text{--}2.5 \mu\text{m}$.

Type locality: Isolated by Dr. S. Udagawa from soil in Tochigi, Japan.

Type: NHL 6086. Isotype: IMI, NY (Lanjoum and Stafleu, 1964). Living cultures of NHL 6086 have been deposited by Dr. S. Udagawa in the

Centraalbureau voor Schimmel-cultures (Baarn, Netherlands).

Conidiophores smooth or roughened, with 1 to 3 septa, $30\text{--}80 \times 1.5\text{--}2.5 \mu\text{m}$; penicilli (fig. 4e) biverticillate, irregularly biverticillate to monoverticillate; metulae $10\text{--}14 \times 2\text{--}3 \mu\text{m}$, 2 to 6 in the verticil; phialides $9\text{--}14$ (–18) $\times 1.8\text{--}2.2 \mu\text{m}$, in verticils of 2 to 6; conidia (fig. 4f) subglobose to elliptical, smooth, hyaline but yellowish green in mass, $2\text{--}3.5 \times 1.5\text{--}2.5 \mu\text{m}$.

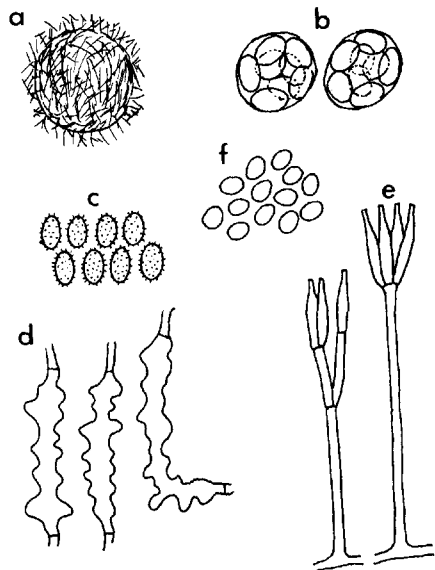
Cultural characters were the same as those described by Udagawa (1966).

Other cultures examined: In addition to the Japanese strain, we also examined a strain which we had isolated from Ohio soil from site K. A comparison of type strain NHL 6086 and the Ohio strain indicates that both represent the same fungus. Culturally, the Ohio strain differs from NHL 6086 in having larger colonies (3.2 cm vs 1.4 cm in diam. in two wk) on Cz, grayish rather than orange colony reverse on Cz and M, and less abundant ascocarps on CM.

As mentioned under *T. flavus* var. *flavus*, *P. ucrainicum* Panassenko was not validly published since no type was designated. Thus, the new combination, *T. ucrainicus* (Panassenko) Udagawa, made by Udagawa for strains NHL 6086 and 6087 obtained from Japanese soil, was also not validly published. Moreover, Udagawa's cultures apparently were misidentified since the ascocarp initials in *T. ucrainicus* (Panassenko) Udagawa and *P. ucrainicum* Panassenko are clearly different. The name *T. ucrainicus* Udagawa, proposed by Stolk and Samson (1972) appears to be validly published for a type was designated and reference was made to the Latin diagnosis of Panassenko. Since *P. ucrainicum* Panassenko has been concluded to be synonymous with *T. flavus* var. *flavus* and therefore cannot be used for the imperfect state of *T. ucrainicus* Udagawa, the name *Penicillium ohiensis* Huang & Schmitt is proposed.

5. *Talaromyces trachyspermus* (Shear) Stolk & Samson Figures 5a-f
= *Talaromyces spiculispurus* (Lehman) (Benjamin, 1955). Conidial state: *Penicillium spiculispurum* Lehman

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FIGURES 5a-f. *Talaromyces trachyspermus*. a. Ascocarp, X70. b. Asci, X2300. c. Ascospores, X2000. d. Ascocarp initials, X1500. e. Detail of penicilli, X1300. f. Conidia, X1700.

FIGURES 6a-f. *Talaromyces wortmannii*. a. Ascocarp, X70. b. Asci, X2000. c. Ascospores, X1700. d. Ascocarp initials, X1600. e. Detail of a penicillus, X1100. f. Conidia, X1600.

Cultures examined.—Isolated from sites A, B, C, F, I, K, M, N.

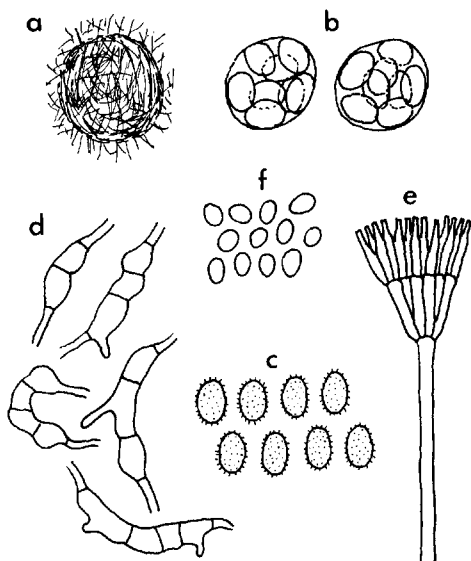
Cultural variations were observed among strains 1103, 1308, 1309b, 1701e, and 1901e of *T. trachyspermus*. On Cz, the colony may be plane (1701e) or wrinkled (1309b), the reverse being brown (1308), yellowish brown (1103), or yellowish (1309b). On M, the colony reverse varies from uncolored (1309b), yellowish (1701e), yellowish brown (1901e), to brown (1308).

6. *Talaromyces udagawae* Stolk & Samson

Conidial state: *Penicillium Udagawae* Stolk & Samson

Isolate 1617 basically agreed with Stolk and Samson's (1972) description of the species, although it differed from the type in having shorter conidiophores (25–80 vs 50–200 μ m) which are smooth or roughened, longer metulae (11–15 vs 7.5–10 μ m), and pinkish buff (XXIX) colonies on Cz which are sudan brown (III) to cinnamon brown (XV) in reverse.

6



Cultures examined.—Isolated from sites C, N.

In *T. udagawae*, Stolk and Samson (1972) observed two types of ascocarp initials: (1) swollen and branched intercalary hyphae, and (2) loosely and irregularly coiled hyphae. We noted only the second type of the initials among Ohio strains. It is possible that the first type represents a later stage in the development of the initials. Besides the loose, irregular coiling of the initials, *T. udagawae* differs from *T. luteus* in having smaller ascospores. The ascocarp initials in the latter consist of one or two regularly coiled hyphae. Despite these differences, the cultural characters and sculpturing of ascospores are remarkably similar in both fungi. Thus, we think that if more strains were examined, *T. udagawae* might prove to be a variety of *T. luteus*.

7. *Talaromyces wortmannii* C. R. Benjamin = *Talaromyces wortmannii* (Klöcker) (Benjamin, 1955).

Conidial state: *Penicillium wortmannii* Klöcker

Cultures examined.—Isolated from sites C, G, H, J, L, N.

Cultural variations have been observed among strains 1021, 1108, 1204, 1601d, and 1901a of *T. wortmannii*. The growth on Cz may be slow (1601d) or fast (1204). The colony may be hyaline (1021) or yellowish (1204), velvet (1204), or floccose (1108), with the reverse being uncolored (1021), yellowish (1901a), brown (1108), or maroon (1204). On M the colony reverse varies from yellowish (1201), light orange yellow (1901a) to purple (1108).

DISCUSSION

The genus *Talaromyces* belongs in the Eurotiaceae. Malloch and Cain (1972) expanded the concept of the Trichocomataceae to include those fungi traditionally placed in the Eurotiaceae. Because the name Trichocomataceae predates Eurotiaceae, Trichocomataceae has been accepted as the valid family for those ascomycetes with *Aspergillus*, *Paecilomyces*, and *Penicillium* imperfect states. In addition to *Hamigera* to which *Talaromyces* is closely related as mentioned in the introduction, *Sporophormis*, recently established by Malloch and Cain (1972), appears to closely resemble *Talaromyces*. However, *Sporophormis* differs from *Talaromyces* in having an *Aspergillus* rather than a *Penicillium* conidial state.

Tremendous cultural variations have been observed among strains of *Talaromyces trachyspermus*, *T. flavus* var. *flavus*, and *T. wortmannii*. Colonies of the latter two fungi are yellowish as are those of *T. helicus* var. *helicus* and *T. ucrainicus*. Culturally, *T. luteus* and *T. udagawae* are distinguished from the above five fungi by their brighter yellowish colonies. It is concluded that characters of the colonies are not reliable features in separating species of *Talaromyces*.

Ascarp initials and ascospore characters, such as spore size, spore shape, and spore markings, are reliable criteria for differentiating species of *Talaromyces*. Because cultures of *Talaromyces* species often fail to produce ascocarps after several cultural transfers, and because

the ascospores are small, the precise identification of the species is sometimes difficult. Consequently, isolates suspected of being *Talaromyces* should be studied and identified immediately after isolation.

From the number of isolates of each species, it is evident that the alcohol isolation method is highly effective in securing *Talaromyces* isolates from soil. By this method not only did we obtain seven species, but we succeeded in isolating each of four species from soils from at least 6 different localities in Ohio. Of the seven species presented, *Talaromyces helicus* var. *helicus*, *T. luteus*, and *T. udagawae* are rarely reported from soil. Satanimi (1971) and Matsushima (1971) recorded the presence of *T. luteus* in soils of Greece and the Solomon Islands, while Smith (1951) and Udagawa (1963) obtained soil isolates of *T. helicus* var. *helicus* from England and Japan, respectively. *Talaromyces udagawae* was described by Stolk and Samson (1972), based on an isolate from Japanese soil. The discovery of *T. luteus* from eight soil samples in Ohio indicates the merit of the selective method we used. It is worth noting that all known species of *Talaromyces* have been isolated from soil.

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